Honey Authenticity Analysis: A Proposed Workflow Using the SCIEX X500R QTOF System

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Importance of Testing for Honey Adulteration

- Honey is one of the most commonly adulterated food commodities globally, especially for high value honeys
  - Dilution of the honey products with a cheaper sugar syrup, such as corn syrup
- Techniques to screen for fraudulent or diluted products include various analytical techniques such as physical, chemical, or morphological assessments
  - Morphological pollen analysis for botanical origin of honey
  - C4 isotope test for the presence of corn-derived sugars.
- Mass spectrometry testing for food authenticity increasing in market
  - Nontargeted mass spectrometry-based methods are employed to find reliable marker compound(s)
  - Targeted methods to test for known adulterants or residues, typically multiple reaction monitoring (MRM)
- This study shows the potential for the X500R QTOF system, SCIEX OS Software, MarkerView™ Software, and MS/MS libraries to be used for investigating honey chemical profiles and potential for adulterant screening.

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SWATH® Acquisition on Honey Samples

- SCIEX X500R QTOF system was used with SWATH Acquisition to collect high resolution MS and MS/MS data on constituents present in the honey samples of different floral origins and honey diluted with corn syrup
- High resolution MS data was used for statistical analyses to evaluate different chemical profiles of the different sample sets
- MS/MS data from SWATH Acquisition allows for further exploration of those chemical profiles - including identifying candidate structural matches through use of spectral libraries

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Sample Preparation

- Honey and corn syrup samples were purchased from local producers in order to best ensure that the samples were authentic in nature and also to capture different floral types of honey products.
- Eleven different honey samples analyzed in triplicate
  - 1 gram of Honey was weighed out
  - 10 milliliters of 50% methanol was added for LC-MS/MS analysis.
- To mimic fraudulent honey products and assess the ability of the method to detect corn syrup adulteration
  - Honey samples were diluted with a series of increasing corn syrup concentrations, up to and including 100% corn syrup by mass, prior to sample extraction.

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Chromatography

- ExionLC™ AD System
- Phenomenex Luna Omega Polar C18 (150×4.6 mm, 3 µm) analytical column
- LC mobile phases:
  - 0.1% formic acid in water (A)
  - 0.1% formic acid in methanol (B)
- Flow-rate of 1 mL/min
- Column temperature of 25 ºC
- Injection volume of 10 µL

Time (min)	B (%)
0	5
38	100
41	100
41.1	5
45	End

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Mass Spectrometry

- Analysis was performed using the SCIEX X500R QTOF System
  - Positive and negative polarity modes
  - MS source conditions were used: CUR=40 psi, CAD=11, IS =5500/-4500 V, TEM=500ºC, GS1=60 psi and GS2=60 psi.
- Variable window SWATH® Acquisition
  - 38 variable sized Q1 windows
  - Accumulation for the TOF MS is 0.1 sec and the accumulation time for the TOF MS/MS is 0.025 sec
- Data were processed using SCIEX OS-MQ 1.5 Software and MarkerView™ Software for statistical analyses
- The SCIEX Natural Products 2.0 Library was used for searching database compound spectra for matches to experimentally derived spectra

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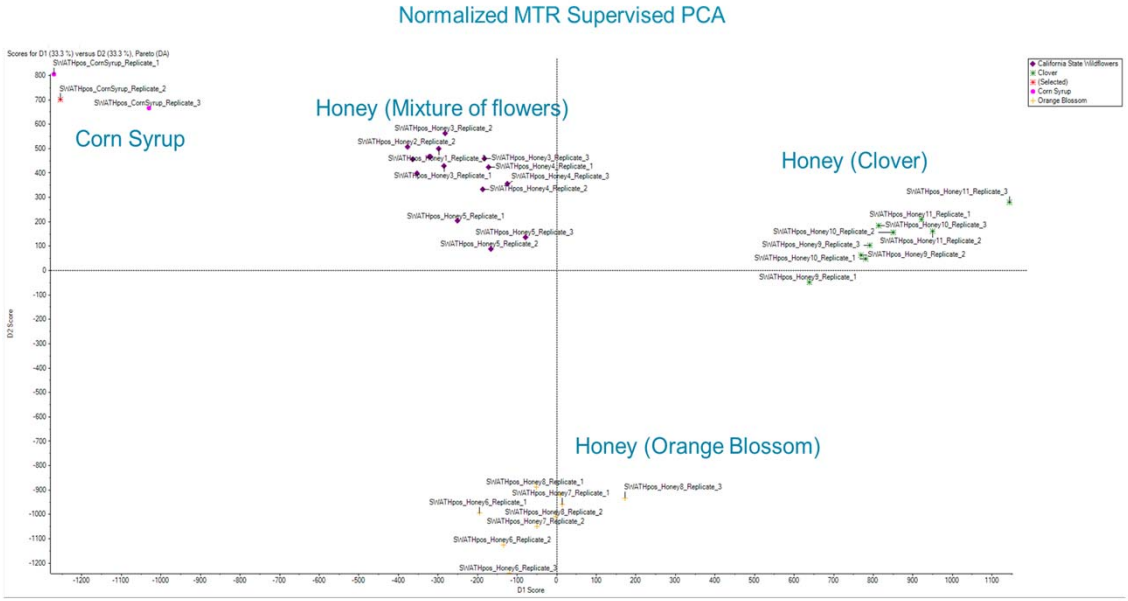
Principal Component Analysis of Honey Variants

QUANTIFICATION ON MS FEATURES

- Different floral origins of honey have different chemical profiles
- Corn syrup clearly separated from all honeys



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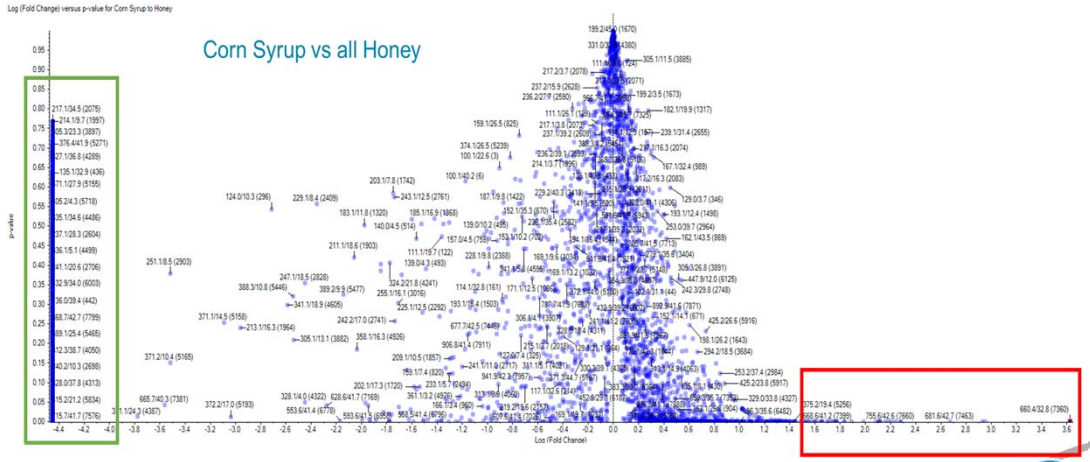
Volcano Plot

T-TEST COMPARISON OF CORN SYRUP VS. ALL HONEYS

- Green - features with the greatest up-regulation in the honeys versus the corn syrup
- Red - features in higher abundance in corn syrup vs. all honey



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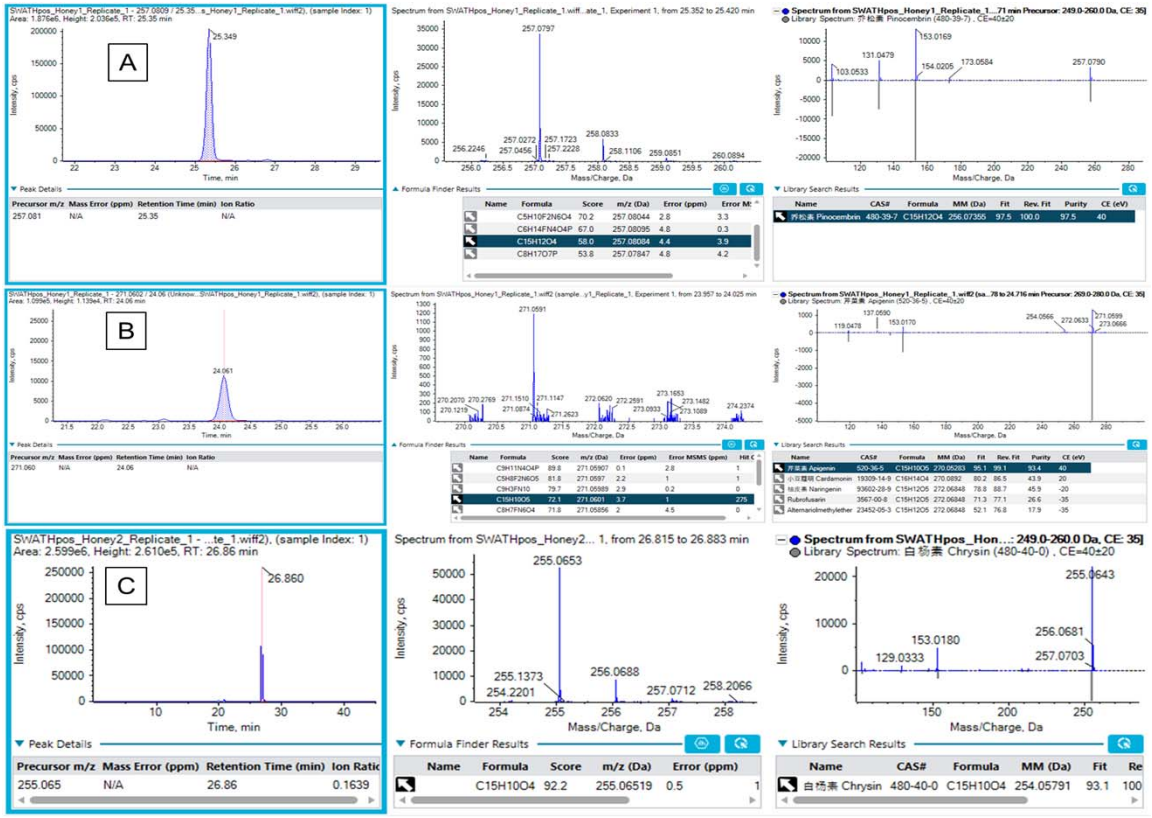
Screening vs. the SCIEX Natural Products Library

IDENTIFICATION FROM SWATH® ACQUISITION MS/MS DATA



Pinocebrin

Purity score 97.5

Apigenin

Purity score 93.4

Chrysin

Purity score 93/1

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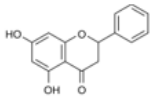
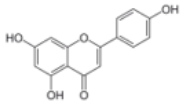
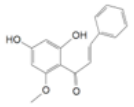
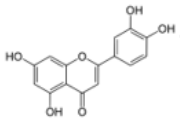
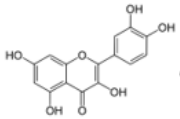
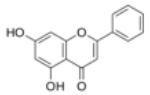
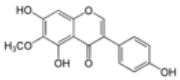
Natural Products Identified in Honeys using MS/MS

Compound Name	Structure	Description	Ref.
Pinocembrin		*Flavanone, antioxidant*	*2*
Apigenin		*Flavone, aglycone of natural glycosides*	*3*
Cardamomin		*Chalconoid*	*5*
Luteolin		*Flavonoid common to fruits, vegeatbles, and herbs used in traditional medicine*	*3*
Quercetin		*Plant flavonoid used as medicine*	*3*
Chrysin		*Flavone found in honey and flowers*	*3*
Tectorigenin		*Isoflavone found in some lilies*	*4*

- Detected in one honey sample with library match >75 and mass error <5ppm
- Polyphenols commonly found in honey

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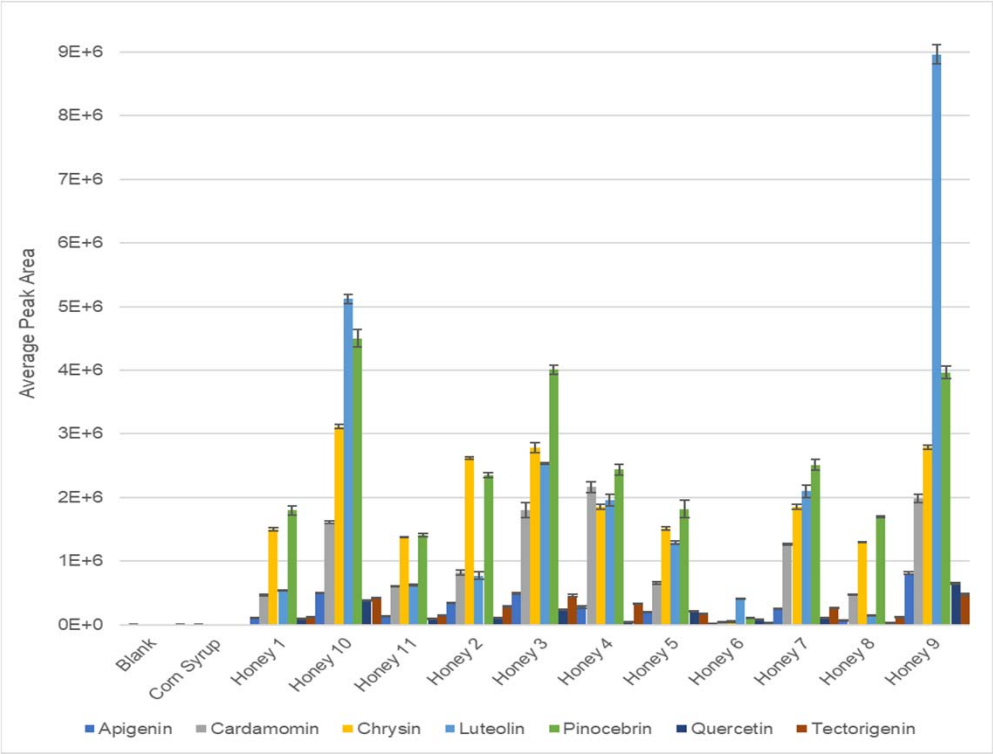
Relative Amounts of Polyphenols

DETECTED IN 11 DIFFERENT HONEY SAMPLES



Apigenin
Cardamomin
Chrysin
Luteolin
Pinocebrin
Quercetin
Tectorigenin

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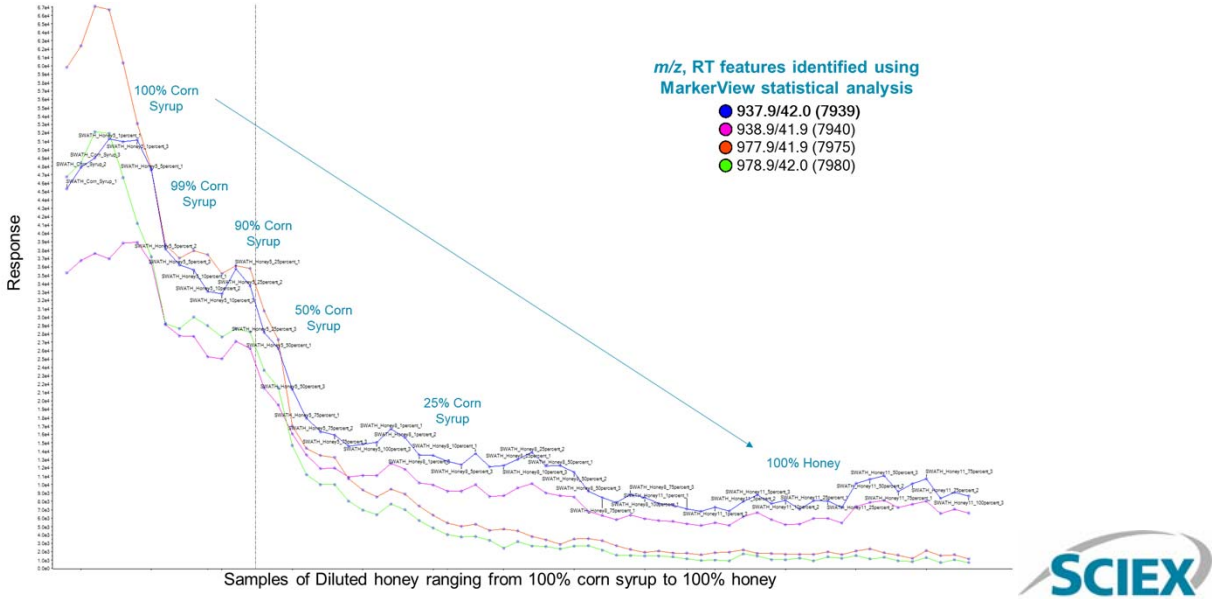
Quantifying Corn Syrup Adulteration in Honey

MARKER COMPOUNDS

- Series of dilutions of a honey sample with corn syrup
- Four mass/retention time features unique to the corn syrup relative to the honey samples (ID not yet known)



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Conclusions

- Non-targeted method using X500R QTOF System, SCIEX OS Software, MarkerView™ Software, and MS/MS libraries for investigating honey chemical profiles and for adulterant screening
  - SWATH® Acquisition allows for the collection of spectral data for all ionizable, detectable constituents in the honey sample
  - Data can be used to profile honey commodities and identify unique chemical markers
  - SCIEX OS Software and SCIEX validated MS/MS library allow for the tentative identification of naturally occurring constituents in honey
  - MarkerView Software with PCA and t-test statistical analyses to find differentiating chemical features in the complex honey and corn syrup matrices
  - Shows that markers unique to corn syrup can be identified and used to screen and quantify dilution of honey products with corn syrup

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References

- Cajka, T., M.R. Showalter, K. Riddellova, O. Fiehn. (2016) *Advances in Mass Spectrometry for Food Authenticity Testing: An Omics Perspective*. In Woodhead Publishing Series in Food Science, Technology and Nutrition, 171-200.

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